Short communication

Effect of feeding frequency on gastric evacuation and the return of appetite in tilapia *Oreochromis niloticus* (L.)

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Abstract

The rate at which food is consumed and the efficiency with which it is utilized are prime factors in determining growth rate. There is a positive relation between growth and feeding frequency. Additionally, feeding frequency is strongly correlated with gastric evacuation time (GET). Nile tilapia *Oreochromis niloticus* were fed to satiation with a pelleted diet at two different feeding frequencies (three meals day^{-1} and five meals day^{-1}) to evaluate the effects of feeding frequency on gastric evacuation and the return of appetite. Ferric oxide was used as an inert indicator to follow gastric evacuation and gastrointestinal (GI) transit. Following an initial meal to satiation with the ferric oxide containing diet, fish were serially dissected at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-prandially. The fish remaining in the treatments receiving three meals day^{-1} were fed to satiation again at 1200 and 1700 h. The fish remaining in the treatments receiving five meals day^{-1} were fed to satiation again at 1000, 1200, 1500, and 1700 h.

Gastric evacuation rates (GER) for both treatments were curvilinear and were best described by a surface-area dependent model. The equation describing GER for fish fed three meals day^{-1} was $V_T=67.0e^{-0.153(x)}$ and for fish fed five meals day^{-1} was $V_T=85.0e^{-0.149(x)}$. The instantaneous evacuation rates obtained by linearizing the data were not significantly different and suggest gastric evacuation is independent of feeding frequency. Fish fed at 4–5-h intervals consume as much as they have evacuated. Fish receiving meals at 2–3-h intervals exhibit gastric overload. The return of appetite following a satiation meal is approximately 4 h in Nile

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tilapia held at 28 °C. Satiation feeding of tilapia at 4-h intervals should increase production efficiency.

1. Introduction

The rate of consumption is a function of environmental conditions, species, dietary composition, meal size, fish size, and feeding frequency (dos Santos and Jobling, 1995; Wang et al., 1998; Liu and Liao, 1999; Riche, 2000) and is one of the prime factors in determining growth rate. In a previous study, it was demonstrated that Nile tilapia (*Oreochromis niloticus*) fed three meals day^{-1} to satiation consumed as much as tilapia fed five meals day^{-1} to satiation (Riche, 2000). Moreover, the fish fed three meals day^{-1} performed better in terms of growth and efficiency.

Feeding intervals or feeding frequency is strongly correlated with gastric evacuation time (Holmgren et al., 1983; Lee et al., 2000), and the return of appetite is closely related to the rate of gastric emptying (Grove and Crawford, 1980; Huebner and Langton, 1982; Lee et al., 2000). Gastric evacuation rate (GER) is also a function of temperature, fish weight, meal size, dietary composition and energy, and feeding frequency (Windell et al., 1969; Grove et al., 1978; Flowerdew and Grove, 1979; Grove and Crawford, 1980; Jobling, 1980; Persson, 1981; Hofer et al., 1982; Holmgren et al., 1983). Although gastric evacuation studies have principally been used for estimating food consumption in the field (Elliott and Persson, 1978; dos Santos and Jobling, 1995), they also manifest themselves for use in aquaculture (Windell et al., 1972; Talbot and Higgins, 1983). It has been demonstrated that the quantity of available food a fish eats is dependent on stomach fullness, and intervals between meals are a function of the rate of emptying (Grove and Crawford, 1980; Holmgren et al., 1983; Grove et al., 1985). Fish culturists can use GER and gastric evacuation time (GET) to develop appropriate feeding strategies for increasing efficiency.

Understanding the rate of digestion and its relationship to GER can allow one to predict the return of appetite under a given set of conditions and diets. Demonstrating a consistent relationship between stomach fullness and appetite return will allow an optimal feeding frequency to be predicted (Grove et al., 1978). Making food available at an appropriate rate and as soon as appetite has returned can maximize intake and increase feed efficiency (Windell et al., 1972; Lee et al., 2000); particularly if automatic feeders are employed (McCarthy et al., 1993; Wang et al., 1998).

Stomach content analysis is often used for determining gastric evacuation, and the use of dyestuffs (Jobling, 1980; Fletcher et al., 1984; Johnston et al., 1994) or other inert indigestible materials (Grove et al., 1978; dos Santos and Jobling, 1991; McCarthy et al., 1993; Marnane and Bellwood, 1997; Saether et al., 1999; Boyce et al., 2000) can also be incorporated to determine GER and gastrointestinal (GI) transit. To conduct these types of studies, samples are collected serially over time by dissection or less invasive techniques

such as X-radiography (Grove et al., 1978; Talbot and Higgins, 1983; McCarthy et al., 1993; Saether et al., 1999), radioactive isotopes (Storebakken et al., 1981), or gastric lavage (dos Santos and Jobling, 1991) and quantitatively assayed for the rate of disappearance from the stomach or the rate of appearance at the GI terminus. However, the rate of disappearance from the stomach is thought to be a better predictor of the return of appetite (Grove and Crawford, 1980). Therefore, the objective of this investigation was to evaluate consumption by *O. niloticus* fed a pelleted diet to satiation at two different feeding frequencies and determine the effects on gastric evacuation and the return of appetite.

2. Methods and materials

2.1. Source of fish

Nile tilapia were obtained from Purdue University and transported to the Michigan State University Fisheries Research Laboratory. Fish were held in a recirculating system at 28 °C and fed a commercial catfish diet during a 4-week acclimation period.

2.2. Experimental design

The experimental system consisted of a 4200-l recirculating system containing eighteen 150-l tanks maintained at 28 °C. Space limitations required the use of three complete blocks. Each block represented a replicate. One fish was stocked into each of the 18 tanks. Individual fish (mean weight 183 g) were considered an experimental unit.

Experimental units were randomly assigned to one of two feeding regimes, and one of eight sample collection periods. The two feeding regimes consisted of feeding to satiation either three times day^{-1} (0800, 1200, and 1700 h), or five times day^{-1} (0800, 1000, 1200, 1500, and 1700 h). The eight sample collection periods were 1, 2, 4, 6, 8, 12, 18, and 24 h following the 0800 meal fed on the experimental day.

The remaining two tanks in each block were used to sample fish at 0 h (before feeding) and 0.5 h following the 0800 feeding. These two sampling periods did not represent treatment differences; therefore, they were not used for statistical analysis but for descriptive purposes only.

Following a 10-day acclimation period during which fish were fed the commercial diet at their assigned feeding times, the fish were fed an experimental diet containing chromium oxide (Cr_2O_3) at 1.0% of the dry diet (Table 1). The diet was formulated to contain 33% crude protein and 13.4 MJ kg^{-1} digestible energy based on predicted values for *O. niloticus* (Anderson et al., 1991; NRC, 1993). The chromium containing diet was fed during a 4-day preliminary period prior to commencement of a 24-h collection period. At initiation of the collection period fish were given one meal at 0800 h of a similar diet containing ferric oxide (Fe_2O_3) at 1.0% of the dry diet. Ferric oxide was substituted into the experimental diet for chromium oxide (Table 1). Fish were fed to apparent satiation at each meal throughout the experiment. Apparent satiation was defined as the point at which a single pellet remained uneaten for 1 min. Consumption during each meal was recorded.

Table 1
Composition of experimental diets fed to *O. niloticus* to determine gastric evacuation rate and intestinal motility

Ingredient	International feed number	Cr_2O_3 diet (g kg^{-1} dry diet)	Fe_2O_3 diet (g kg^{-1} dry diet)
Herring meal	5-02-000	259.7	259.7
Soybean meal (solvent extracted)	5-02-612	277.5	277.5
Wheat bran	4-05-190	100.0	100.0
Dextrin	4-08-023	100.0	100.0
Mineral premix[a]		60.0	60.0
Vitamin premix[b]		3.0	3.0
Carboxymethyl cellulose		20.0	20.0
α-Cellulose		68.0	68.0
Ascorbic acid		1.0	1.0
Choline chloride		0.8	0.8
Menhaden oil	7-08-049	75.0	75.0
Soy oil	4-07-983	25.0	25.0
Chromic oxide		10.0	–
Ferric oxide		–	10.0
Total		1,000.0	1,000.0

[a] Mineral premix contained (g kg^{-1} dry mix): $CaSO_4$, 350.0; NaH_2PO_4, 250.0; KH_2PO_4, 250.0; $MgCO_3.5H_2O$, 20.0; $ZnSO_4.7H_2O$, 3.0; $FeSO_4.7H_2O$, 2.8; $MnSO_4.H_2O$, 2.0; $CuCl_2.2H_2O$, 1.0; $AlCl_3.6H_2O$, 1.0; KF, 0.5; KI, 0.1; Na_2SeO_3, 0.1; $CoCl_2.6H_2O$, 0.1; $NaMoO_4.2H_2O$, 0.1.

[b] Warmwater fish performance premix (Hoffmann-La Roche, Nutley, NJ)—as incorporated in the diet: vitamin A, 10,582 IU; vitamin D3, 2381 IU; vitamin E, 132 IU; vitamin K, 2 mg; B_{12} μg, 4.4 μg; folic acid, 5.3 mg; riboflavin, 17.2 mg; pantothenic acid, 42.3 mg; niacin, 105.8 mg; choline-Cl, 529.1 mg; thiamin, 11.9 mg; pyridoxine, 13.2 mg; biotin, 165 μg.

Fish were serially dissected at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h following the 0800 h feeding during the 24-h collection period (Fig. 1). Fish were euthanized in tricaine methanesulfonate at a concentration of 500 mg l^{-1} before dissection. The fish remaining in the treatments receiving three meals day^{-1} were fed to satiation again at 1200 and 1700 h. The fish remaining in the treatments receiving five meals day^{-1} were fed to satiation again at 1000, 1200, 1500, and 1700 h (Fig. 1). Fish were not fed prior to dissection when
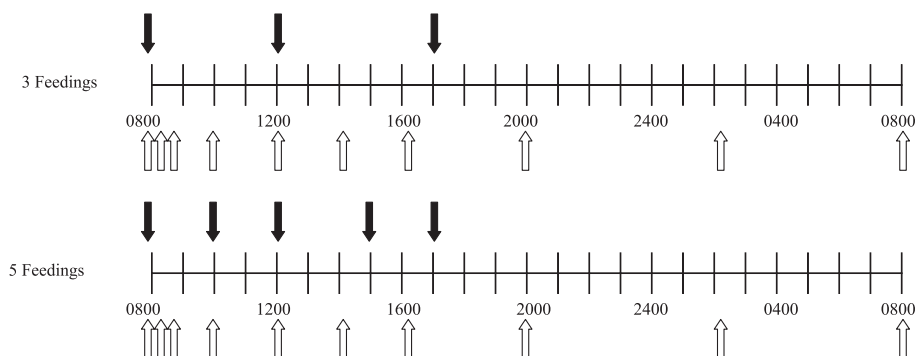

Fig. 1. Feeding times (black arrows) and sampling times (white arrows) for the 24-h collection period.

feeding and sampling times coincided. After the initial feeding with the ferric oxide diet, all subsequent feedings were with the chromium oxide diet.

The GI tract was excised and rinsed with cold distilled deionized water (DDI). Visceral fat and other tissues were removed. The GI tract was divided into seven segments consisting of the stomach, and two equal size segments each from the anterior, middle, and posterior intestine. The excised segments were visually inspected for iron containing digesta. Undigested feed and feces from each segment were rinsed with cold DDI water. The samples were dried at 105 °C for 24 h. Dried feces were ground and mixed prior to analysis.

2.3. Iron analysis

Ground samples, approximately 100 mg, were wet ashed in 5 ml concentrated sulfuric acid until charred. Charred samples were allowed to cool slightly before adding 30% hydrogen peroxide drop-wise until all carbonaceous material was oxidized. Samples were reheated for 5 min and allowed to cool slightly. Following the addition of 5 ml DDI, samples were reheated for 2 min. Samples were then brought to 100 ml with DDI and filtered through Whatman #1 filter paper.

Filtered samples were analyzed for iron colorimetrically at $\lambda=535$nm (Davies et al., 1972). The assay was slightly modified by substituting bathophenanthroline disulfonic acid for 4,7-diphenyl-1,10-phenanthroline thereby obviating the need for a sulfonation step.

2.4. Statistical analysis

Both the GER and the rate of iron appearance at the GI terminus were evaluated. Iron analysis of each segment was performed on fish sampled at time zero to correct for background iron. ANOVA using the general linear method (SAS, 1979) was performed on iron consumption, total consumption, and amount of iron appearing in the terminal segment of the posterior intestine. The data were analyzed as a randomized complete block design with all factors fixed. Contrasts between the two feeding regimes were performed on iron appearing in the terminal segment of the posterior intestine.

Data utilized for determining GER were transformed. The transformed data set (natural log cumulative iron) was plotted versus time to generate a linear relationship. Differences between the slopes were analyzed by *t*-test. Similarly, a *t*-test was used to test the slopes defining the relationship between rate of iron appearance in the terminal segment and time following initial feeding. Significance was reported at $P<0.05$.

3. Results

Mean consumption of the iron marker containing diet was 1.06 and 1.03 g for the treatments fed three meals day^{-1} and five meals day^{-1}, respectively. Mean total consumption by fish receiving the full days complement of feed was 1.8% and 2.1% body weight for the treatments fed three meals day^{-1} and five meals day^{-1}, respectively.
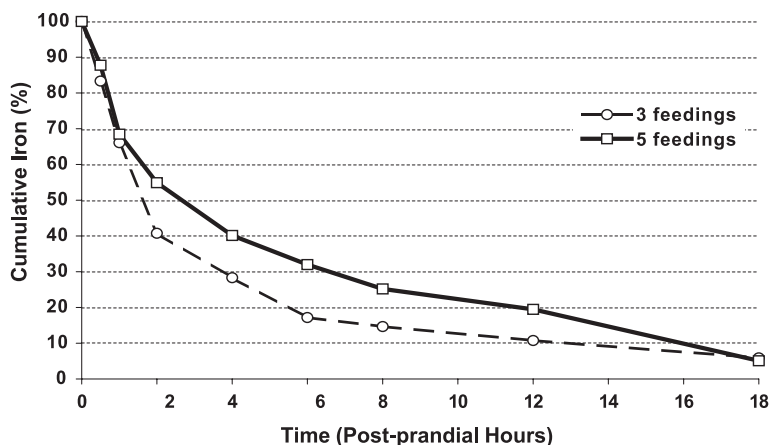
Fig. 2. Rate of gastric evacuation in *O. niloticus* fed three meals day^{-1} or five meals day^{-1} to satiation. Cumulative iron is relative to the total gastric iron collected over 24 h. The rates were $67.0e^{-0.153(x)}$, R^2=0.90 (three meals day^{-1}); and $85.0e^{-0.149(x)}$, R^2=0.97 (five meals day^{-1}).

There were no significant differences in iron consumption or total consumption between the treatments. Few fish dissected at time 0 (prior to feeding) had digesta remaining from the previous day's feeding. The digesta that was found was principally localized in segments 5–7. No digesta was found in the gastric region of any fish. Where digesta was found, it was analyzed for iron and the results used to correct for background iron.

The contrast between the green chromic oxide marker and red ferric oxide marker was readily discernable during dissection. Iron was observed throughout the GI tract at 2 h following feeding with the iron containing diet. At 4 h following the initial feeding, a red/green mixed bolus was noted in segment 4, and at 6 h the mixed bolus was observed in
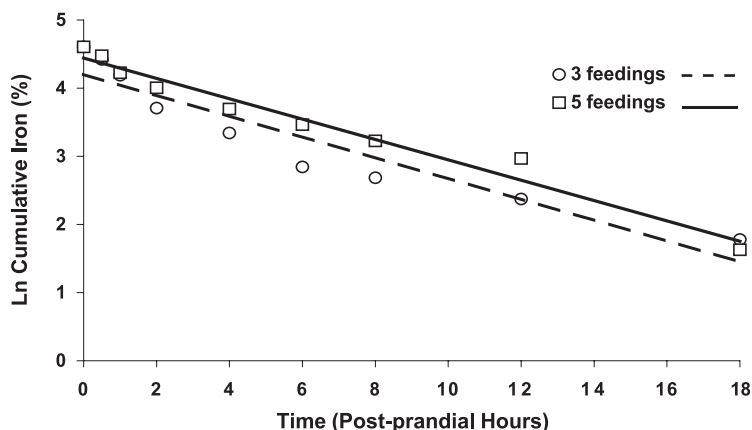

Fig. 3. Semilogarithmic plot of the instantaneous rate of evacuation in *O. niloticus* fed to satiation three times day^{-1} or five times day^{-1}. The slopes −0.153 (three feedings) and −0.148 (five feedings) were not significantly different.
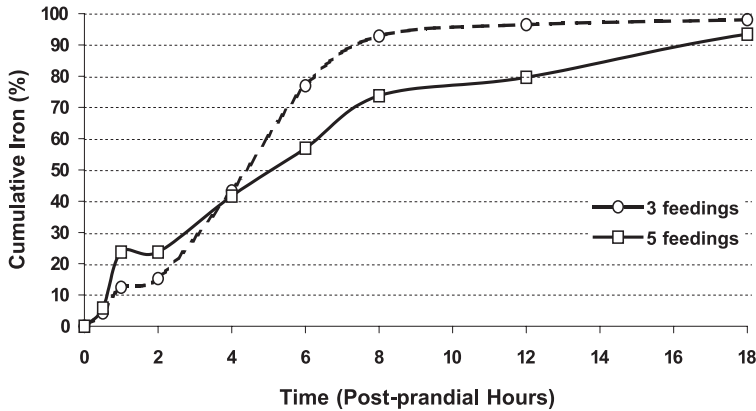
Fig. 4. Rate of iron appearance at the intestinal tract terminus of *O. niloticus* fed to satiation three times day[−1] or five times day[−1]. Cumulative iron is relative to the total iron collected at the terminus over 24 h.

the terminal segment for both treatments. At 24 h, the stomachs in fish fed five meals day[−1] contained some of the mixed red and green digesta, whereas the stomachs in fish fed three meals day[−1] were empty and flaccid. Visual inspections were verified by analytical determination.

The initial rate of gastric emptying for both treatments was similar during the first hour (Fig. 2). Following the first hour, GER of fish fed five meals day[−1] was slower than that of fish fed three meals day[−1]. Gastric evacuation rates for both treatments were curvilinear and best described by the exponential function $V_T = V_0 e^{-b(x)}$ where V_T = volume of feed at time T, V_0 = volume of feed at time 0, b = the instantaneous evacuation rate, and x = time post-prandial. The equation describing GER for fish fed three meals day[−1] was $V_T = 67.0 e^{-0.153(x)}$ with a correlation coefficient of $R^2 = 0.90$, and for fish fed five meals day[−1] was $V_T = 85.0 e^{-0.149(x)}$ with a correlation coefficient of $R^2 = 0.97$. A *t*-test following natural
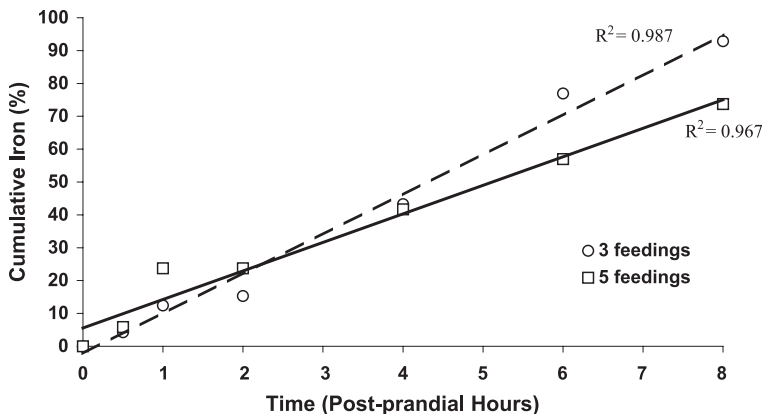

Fig. 5. Iron appearance during the first 8 h at the terminal segment of the intestinal tract of *O. niloticus* fed to satiation three times day[−1] or five times day[−1]. Cumulative iron is relative to total iron collected over 24 h.

log transformation of cumulative iron, representing the instantaneous evacuation rate, suggested that the slopes were not significantly different (Fig. 3).

The rate at which iron appeared at the terminal segment of the intestine was slightly more rapid for fish fed five meals day^{-1} over the first hour (Fig. 4). The amount of iron appearing in the terminal segment between the 2-h sampling and the 4-h sampling and between the 4-h sampling and the 6-h sampling was significantly higher in the group fed three meals day^{-1}. Conversely, the increase between the 12- and 18-h samplings was significantly higher in the group fed five meals day^{-1}.

The rate at which iron appeared at the terminus was linear over the first 8 h post-prandially (Fig. 5). A slope analysis indicated that the rates were not significantly different. The time required for 90% of the recovered iron to appear at the terminus in fish fed three meals day^{-1} was 8 h, and for fish fed five meals day^{-1} was 18 h.

4. Discussion

A number of mathematical models exist to describe GER and GET (Jobling, 1981, 1986a; Persson, 1986). Some of these models attempt to describe an initial lag phase attributed to the time required for sufficient physical and chemical breakdown of the ingested feed to pass the pyloric sphincter.

We did not detect an initial emptying delay with our sampling scheme. Initial emptying delays are a function of feed quality, quantity, temperature, and size of the feed particles all of which effect the rate of physical, chemical and enzymatic digestion (MacDonald et al., 1982; Fletcher et al., 1984; Jobling, 1987; Salvanes et al., 1995). The first sampling period was at 0.5 h following the first meal. In the dab *Limanda limanda* held at 15 °C, Fletcher et al. (1984) observed a delay of approximately 1 h. Therefore, it is possible the fish in this experiment held at 28 °C also exhibited a short delay that was not detected due to the timing of our first sampling period. Additionally, friable pellets may not have the properties required for prolonged gastric retention (Jobling, 1986b). Since the pellets offered were relatively small and homogenous with well-ground and highly digestible materials, it is possible that there was an initial delay less than 0.5 h. Because of this inability to detect a lag time, we did not try to fit the data to a logistic curve which is suitable if there is an initial lag time (MacDonald et al., 1982; Bromley, 1987). Moreover, Bromley (1987) suggests feeds that disintegrate rapidly tend to be evacuated in an exponential fashion.

Exponential, rectilinear, square root, and surface-area models of GER all indicate that evacuation is fastest initially and slows down as stomach fullness declines (Bromley, 1987). However, the most widely used are the volume-dependent and surface-area-dependent models. Both describe a curvilinear relationship, but differ in their assumptions (Jobling, 1981; Persson, 1986).

With volume-dependent models, the volume of feed ingested, through distension of the stomach, modifies the rate at which food is evacuated (Jobling, 1981). The assumption is distension of the stomach initiates peristaltic contraction and the circumferential tension is proportional to the radius, and therefore proportional to the square root of the volume of the residuum (Jobling, 1981, 1986a). The larger the original volume, the greater is the

initial rate of emptying. With this model, gastric evacuation curves are linearized by square root transformation of stomach residuum and plotted against post-prandial time.

With surface-area-dependent models, it is believed that the surface area of the food influences digestion and evacuation. Digestive enzymes attack the outer surface of the food and the rate of digestion is proportional to the particle surface area (Jobling, 1981, 1986a; Grove et al., 1985). The rate of evacuation is predicted to be faster for a meal consisting of small food particles than large particles (Persson, 1986; Jobling, 1987). With this model, gastric evacuation curves are linearized by an exponential transformation of stomach residuum and plotted against post-prandial time.

Plotting the gastric evacuation curves for the data obtained in this study indicates curvilinear relationships (Fig. 2). Without an a priori justification for preferring one model over the other, the data from these gastric evacuation curves were subjected to both square root and natural log transformation. The transformed data were plotted against post-prandial time, and the two models subjected to least squares estimates of the residuals. Both feeding frequencies were best described by the exponential function $V_T = V_0 e^{-b(x)}$. This relationship has also been demonstrated in the tilapia *Sarotherodon mossambicus* (De Silva and Owoyemi, 1983), and a diversity of other species (El-Shamy, 1976; Grove and Crawford, 1980; Persson, 1981; Huebner and Langton, 1982; Talbot and Higgins, 1983; Bromley, 1987; Saether et al., 1999).

Jobling (1987) suggests that evacuation of dry pelleted feeds is best described by a square root or linear function; however, this was not supported by our data. Enzyme activity is an exponential function and is affected by temperature. Therefore, it is possible that the exponential function is more appropriate for pellet fed tilapia reared in warmer temperatures (Brodeur, 1984), or because of a smaller stomach storage capacity (Jobling and Davies, 1979) than the species evaluated by Jobling (1987).

The slopes described by the term *b* represent the instantaneous rate of gastric evacuation (Jobling, 1981). The instantaneous rates for the two feeding frequencies in this study were similar at -0.153 (three meals day^{-1}) and -0.149 (five meals day^{-1}), and were not significantly different from each other (Fig. 3). The data suggest that evacuation rate is independent of feeding frequency. Moreover, the slopes suggest despite more frequent feedings the rate of energy flow from the stomach is relatively constant (Jobling, 1980).

The validity of the model hinges on two assumptions. The first assumption is the ferric oxide dye incorporated functions as a suitable marker. According to Kotb and Luckey (1972), ferric oxide may violate two of the criteria for defining a suitable marker. These two criteria are: (1) the compound must be inert with no toxicological effects; and (2) neither absorbed nor metabolized within the GI tract. Although these factors may suggest that iron is unsuitable for digestibility studies, neither one should preclude the use of ferric oxide for tracking GI passage or gastric evacuation in fish.

Iron toxicity has been demonstrated in domestic animals. Maximum tolerable dietary levels range from 500 mg kg^{-1} in sheep to 3000 mg kg^{-1} in swine (NRC, 1980), and is 1380 mg kg^{-1} in rainbow trout *Oncorhynchus mykiss* (Desjardins et al., 1987). However, these maximum tolerable levels are based on long-term feeding studies that utilized iron sources with high biological availability. In contrast to the more available ferrous state, we incorporated the ferric state that is very little if at all absorbed by fish (Maage and Sveier, 1998; Bury et al., 2001).

In mammals, dietary Fe^{3+} is utilized following reduction, principally via a membrane bound ferric reductase and subsequently enters the enterocytes via a pH-dependent Fe^{2+}/H^+ cotransporter (Bury et al., 2001). Although evidence exists to suggest a similar cotransport mechanism in fish, the level or functional capacity of a membrane ferric reductase is negligible making active uptake of Fe^{3+} doubtful (Bury et al., 2001). Moreover, the dominant site for iron absorption in fish is the posterior intestine (Bury et al., 2001), suggesting ferric oxide is acceptable for evaluating gastric evacuation.

Additionally, the calculated total dietary iron was 7151 mg kg^{-1} of the dry diet, 89 mg contributed by dietary ingredients (Hertrampf and Piedad-Pascual, 2000), 62 mg contributed by the mineral supplement (Table 1), and 7000 mg contributed as ferric oxide. Although there are no known mechanisms for uptake of Fe^{3+} in fish (Bury et al., 2001), it is possible some Fe^{3+} losses occurred via reduction to Fe^{2+} as a result of interactions with other dietary components, i.e. ascorbic acid. However, the method of Davies et al. (1972) quantifies total iron regardless of its initial valence state. Moreover, any loss across the mucosa following reduction would be inconsequential relative to the amount ingested and traversing the GI tract. Since the dietary history, diet composition, iron consumption, and iron status of the fish were the same for the two treatments, any small losses could be considered a systematic error without consequence to the results (De Silva and Owoyemi, 1983). Despite these complicating factors, feeding studies utilizing iron and iron compounds as markers have been conducted with terrestrial animals (Kotb and Luckey, 1972; Teeter, 1985), Atlantic salmon *Salmo salar* (Talbot and Higgins, 1983), rainbow trout (Kabir et al., 1998), and tilapia (Barash et al., 1984).

The second assumption is that 95% evacuation is a reasonable practical limit for determining GER and GET in a culture setting. Since there is no end-point on a semilogarithmic plot, a practical limit must be applied (Grove and Crawford, 1980). Although determining the point at which the stomach contains zero residuum warrants merit, the objective of this study was to understand the relationship between feeding frequency, stomach fullness, and the return of appetite. Therefore, we felt that 95% evacuation was a practical endpoint to consider the gastric residuum as zero. This was achieved at 18 h following the first feeding (Fig. 3). Elliott and Persson (1978) used 99% evacuation, but the remaining residuum at 18 h in this study was still below the level deemed by Bromley (1987) as acceptable for classifying the stomach as empty.

Mean fish weight, diet, temperature, amount to satiation, and particle sizes were the same between the two treatments. The only factor altered was the frequency at which the two treatments were fed. The instantaneous rate of evacuation would therefore be expected to be the same, as the results suggest (Fig. 3).

The two curves are offset by the difference in the constant V_0, which represents the maximum volume at time 0. The model suggests that fish fed five feedings day^{-1} had a higher maximum volume. However, maximum stomach volume is proportional to weight (Flowerdew and Grove, 1979; Jobling, 1980; Holmgren et al., 1983). Since fish between the two treatments were the same weight, maximum volume would be predicted to be the same. The likely explanation for this incongruity is that fish fed five feedings day^{-1} were fed a subsequent meal 2 h following the first meal. This corresponds to the point where the two gastric evacuation curves begin to deviate. The affect was to fill the stomach again and shift the gastric evacuation curve to resemble an increased meal size at the first feeding.

This would explain the higher intercept value for the slope of the fish receiving five feedings day^{-1} (Jobling, 1980).

A curvilinear relationship would predict a faster evacuation of the gastric contents with multiple feedings. Each subsequent meal increases the stomach volume and the initial evacuation following a feeding is the most rapid. The rate of evacuation is faster with the greater stomach volume in dab (Fletcher et al., 1984), and catfish *Ictalurus punctatus* (Andrews and Page, 1975), but not in bluegill *Lepomis macrochirus* (El-Shamy, 1976) or turbot *Scophthalmus maximus* (Bromley, 1987). Additionally, multiple meals have been shown to accelerate (Tyler, 1970; Persson, 1984; Andersen, 1998) or slow down (El-Shamy, 1976; Fletcher et al., 1984; dos Santos and Jobling, 1991) gastric evacuation.

In the present study, the rate of evacuation appeared to slow for the treatment receiving the extra meal (Fig. 2) although the instantaneous evacuation rates were not different. This observation was further supported by the slower rate of appearance of iron at the terminus of the GI tract (Fig. 4). Moreover, plotting the mean percent iron recovery at the GI terminus over the 24-h experimental period indicates the peak recovery of iron in fish fed three times day^{-1} occurred 2 h earlier than in fish fed five times day^{-1} (Fig. 6). El-Shamy (1976) also observed a decreased evacuation in bluegill fed multiple meals relative to fish fed one meal, although the decrease, as in this study, was not significant.

In some species, food ingested leaves the stomach on a first in–first out basis (Fletcher et al., 1984; Grove et al., 1985; Bromley, 1987). Ingested food retained in the stomach accumulates in the fundic region where it comes in contact with acid-secreting cells localized to the ventral face of the mucosa. However, this is not always the case with tilapia. Tilapia possess the ability to by-pass the fundic region of the stomach with food passing directly from the esophagus to the pylorus and into the intestine (Moriarity, 1973). However, ingested food by-passing the stomach enters the intestine without the benefit of initial hydrolysis and mixing (Moriarity, 1973).

As gastric evacuation is incomplete 2 h following the first feeding, it is likely that some of the newly ingested food given to fish receiving five feedings day^{-1} passes to the
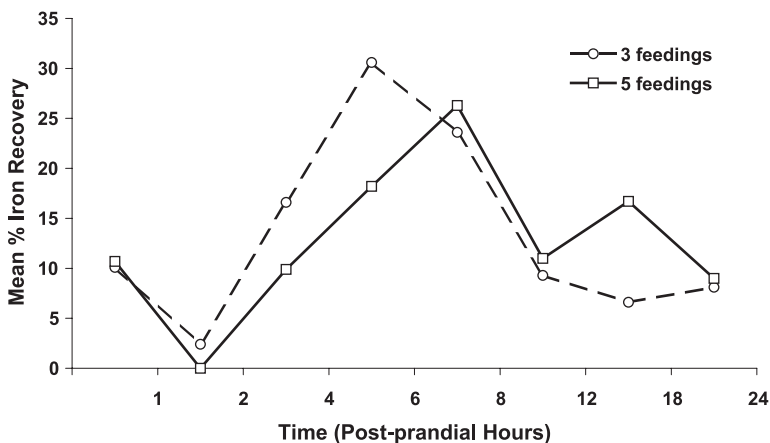

Fig. 6. The mean percent of total iron recovered from the GI tract over the 24-h sampling period.

intestine. Food passing directly into the intestine undergoes inefficient digestion resulting in lower utilization efficiency for the same level of consumption. In a previous study, fish fed five meals day^{-1} consumed as much as those fed three meals day^{-1} but were less efficient in converting nutrients (Riche, 2000). Additionally, Gwyther and Grove (1981) determined that there is a significant positive correlation between feeding frequency and stomach emptying time. GER increases with more frequent feedings leading to decreased digestion efficiency (Powell, 1972; Tsevis et al., 1992). There are other reports of less efficient digestion and utilization in species fed at short intervals (Thia-Eng and Seng-Keh, 1978; Tsevis et al., 1992; Liu and Liao, 1999).

Alternatively, it is possible that feed from the initial feeding is unable to pass to the intestine because of the newly ingested feed. In this case, it would appear as though the feed were being evacuated more slowly. This was suggested as the cause for impeded evacuation in cod *Gadus morhua* (dos Santos and Jobling, 1991) and polar cod *Boreogadus saida* (Saether et al., 1999) fed multiple meals. The feed ingested 2 h following the initial feeding with the ferric oxide containing diet then would not be following the first in–first out dictum. A greater portion of ingested ferric iron would remain in the stomach to be released at a later time. A plot of gastric iron expressed as a percent of total tract iron at each sampling time indicates the percent as gastric iron drops precipitously until the second meal is introduced (Fig. 7). This suggests the initial meal containing iron is not passing to the intestine on a first in–first out basis. Moreover, fish fed five meals day^{-1} were observed to have residual ferric iron in the stomach at 24 h where those fed three meals day^{-1} did not. This is similar to the observations of dos Santos and Jobling (1991) who described this phenomenon as a "stacking of meals" in fish fed multiple meals.

It was estimated in rainbow trout that it requires 15 h to evacuate a 1% BW meal (Grove et al., 1978). According to their evacuation curve, it was estimated that 80–90% evacuation would require 6 h, which the authors suggested corresponded to the return of
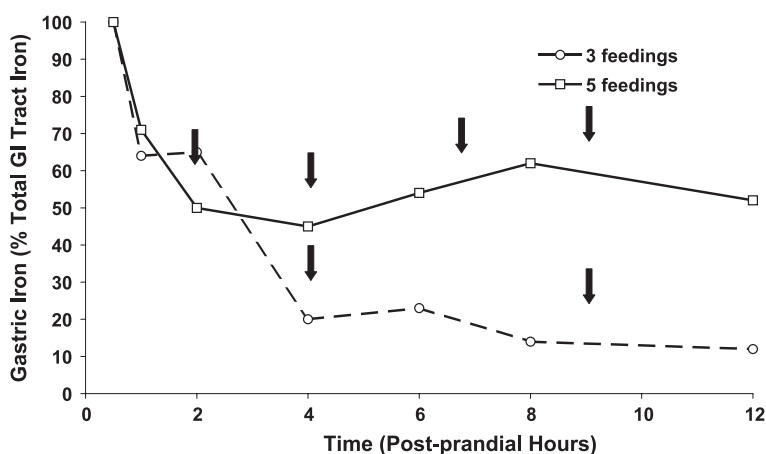

Fig. 7. Percent of total GI tract iron as gastric iron in *O. niloticus* fed to satiation with a ferric oxide containing diet. Arrows indicate subsequent feedings to satiation.

appetite. In the stomachless *Blennius pholis*, return of appetite was estimated at $4-15$ h depending on the size of fish (Grove and Crawford, 1980). The evacuation curves constructed from the data collected in this study would predict 8 h are required to attain 80% evacuation.

It has been suggested that the amount of food consumed at a second meal is equivalent to the amount of food already digested (Huebner and Langton, 1982). Following the technique of Grove et al. (1985), we applied the predicted evacuation, obtained from instantaneous evacuation rates to consumption data obtained under similar conditions (Riche, 2000). Fish receiving meals at 2-, 3-, 4-, or 5-h intervals are predicted to have evacuated 27%, 36%, 64%, and 70% of their initial meal to satiation, respectively (Table 2). Conversely, during their ensuing meal to satiation, they consumed the equivalent of 52%, 58%, 73%, and 69% of their original meal, respectively (Table 2). Fish fed at $4-5$-h intervals consume as much as they have evacuated. Fish receiving meals at $2-3$-h intervals are evacuating more quickly than predicted, or feed is entering the intestine without sufficient gastric digestion. In either case, such a gastric overload would reduce absorption efficiency (Jobling, 1986b). Moreover, feeding tilapia at $4-5$-h intervals instead of $2-3$-h intervals represents a potential savings in time and labor.

Seymour (1989) suggested the optimal feeding frequency is one in which the feeding interval corresponds to the volume and rate of emptying of the stomach. If the optimum interval between feedings is the point at which evacuation of the previous meal is matched by consumption as in winter flounder (Huebner and Langton, 1982) and European eels *Anguilla anguilla* (Seymour, 1989), then the optimum interval between feedings for *O. niloticus* at $28\,^{\circ}\text{C}$ is $4-5$ h, depending on energy and composition of the diet. Moreover, due to the higher energy requirements of small fish, it is possible that the return of appetite in tilapia less than 20 g is shorter (NRC, 1993).

These findings support previous work investigating the effects of feeding frequency on growth and efficiency in tilapia (Riche, 2000). However, this is slightly longer than the previously reported food passage rate of $2.5-3.0$ h for the tilapia *O. aureus* (Popma, 1982). The shorter food passage rates observed by Popma (1982) may be due to differential passage rates of algae relative to pellets, higher rearing temperatures, or may

Table 2
Hours between feedings, predicted gastric evacuation (%) from evacuation curves, and consumption[a] at the ensuing meal as a percent of satiation

Hours between meals	Predicted gastric evacuation(%)	Consumption (%Satiation)
3 Feedings		
4	64	73
5	70	69
5 Feedings		
2	27	53
2	27	49
3	36	58
2	27	55

[a] From Riche, 2000.

represent true species differences. Further research is required to evaluate the return of appetency in other tilapia species and for different size classes.

5. Conclusion

The present study shows that ferric oxide can be used as a marker to follow gastric evacuation and gastrointestinal transit in fish. Nile tilapia fed multiple meals to satiation with a pelleted feed evacuate in a curvilinear fashion best described by an exponential model. The instantaneous evacuation rate obtained by linearizing the data suggests that gastric evacuation is independent of feeding frequency in Nile tilapia. However, feeding at intervals shorter than the time required for the return of appetite can lead to gastric overload resulting in reduced absorption efficiency. The return of appetite following a satiation meal, defined as the point that consumption is equivalent to the amount of the previous meal evacuated, is approximately 4 h in Nile tilapia held at 28 °C.

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